the hybridized probes are analyzed in a mass spectrometer and the position of the probes on the sample holder permits a classification of the hybridizing DNA sample (Abstract, Examples 1-2, Figures 10-11, Column 4, lines 25-55, and claim 1)." The Patent Office then concludes by stating the following:

> It would have been prima facie obvious to one having ordinary skill in the art at the time the invention was made to combine and substitute the DNA diagnostic based on mass spectrometry of Koster in the method of detection of methylated nucleic acid using agents which modify unmethylated cytosine and distinguish modified methylated and unmethylated nucleic acids of Herman et al. since Koster states, "In addition, because the instant disclosed processes allow the nucleic acid fragments to be identified and detected at the same time by their specific molecular weights (an unambiguous physical standard), the disclosed processes are also much more accurate and reliable than currently available procedures (Column 4, lines 50-55)." An ordinary practitioner would have been motivated to combine and substitute the DNA diagnostic based on mass spectrometry of Koster in the method of detection of methylated nucleic acid using agents which modify unmethylated cytosine and distinguish modified methylated and unmethylated nucleic acids of Herman et al. in order to achieve the express advantages, as noted by Koster, of processes which allow the nucleic acid fragments to be identified and detected at the same time by their specific molecular weights (an unambiguous physical standard), and which are also much more accurate and reliable than currently available procedures.

Applicant respectfully traverses the foregoing rejection.

Claim 1, from which claims 2-5 and 7-23 depend, recites "[a] method for identifying cytosine methylation patterns in genomic DNA samples, said method comprising the steps of:

a) chemically treating a genomic DNA sample in such a way that cytosine and 5-methylcytosine react differently and a different base pairing behavior of the two products is obtained in the duplex;

b) enzymatically amplifying portions of the thus-treated DNA sample;

c) binding the amplified portions of the thus-treated DNA sample to a surface;

d) contacting a set of probes of different nucleobase sequences, each of which contains the dinucleotide sequence 5'-CpG-3' at least once, to the immobilized DNA samples for hybridization;

e) removing any non-hybridized probes from the immobilized DNA samples;

f) analyzing the hybridized probes in a mass spectrometer, wherein the position of the hybridized probes on the surface permits a classification of the immobilized DNA sample hybridized thereto;

g) assigning a peak pattern obtained from the mass spectra to a methylation pattern for the immobilized DNA and comparing the peak pattern with a database to identify cytosine methylation patterns in the genomic DNA sample."

The foregoing rejection is predicated, at least in part, on the Patent Office's contention that Herman et al. teaches steps (a) through (e) of the method of claim 1. However, as explained below, such a contention is in error.

Herman et al. relates to a technique referred to therein as "methylation specific PCR" or MSP. According to MSP, unmethylated cytosine, but not methylated cytosine, is converted to uracil, and primers specific **only for methylated nucleic acids**, as opposed to unmethylated nucleic acids, are used to amplify the methylated nucleic acids. **Consequently, the Herman et al. MSP method results in only the methylated fragments being amplified** as the unmethylated fragments do not undergo any amplification. This is why Herman et al. repeatedly characterizes its technique as involving "the use of PCR as the step to distinguish methylated from unmethylated DNA." (See e.g., col. 4, line 65 through col. 5, line 1; col. 5, lines 43-46; and col. 6, lines 3-6, of Herman et al..)

By contrast, the claimed method is distinguishable from the MSP method of Herman et al. in that the claimed method does not use PCR to **distinguish or select** methylated DNA from unmethylated DNA. Instead, the present invention involves amplifying **both** methylated and unmethylated fragments and, **thereafter**, hybridizing a set of probes to the already amplified DNA for the purpose of detecting methylation therein.

In other words, whereas Herman et al. involves **selectively amplifying methylated DNA**, the claimed invention involves amplifying **both** methylated and unmethylated fragments and, **thereafter**, hybridizing a set of methylation-specific probes to the already amplified DNA for the purpose of detecting methylation therein.

In view of the above, it can readily be appreciated that the claimed step (d) of hybridizing methylation-specific probes to the immobilized amplificates is neither taught nor suggested by Herman et al.. In fact, it would not make any sense to use such probes in MSP since, in MSP, the step of generating the amplificates is already selective for methylation, and no additional information would be imparted by the use of such probes. Similarly, the claimed step (e) of removing any non-hybridized probes from the immobilized DNA samples is neither taught nor suggested by Herman et al. since there would be no reason to perform step (e) in MSP. As noted above, this is because all of the MSP amplificates are inherently indicative of methylation; therefore, there would be no reason to hybridize any probes to the MSP amplificates for the purpose of indicating methylation and there would be no reason to remove any non-hybridized probes.

Köster, which relates to a mass spectrometer-based process for detecting a particular nucleic acid sequence in a biological sample, fails to cure the above-noted deficiencies of Herman et al.. Moreover, for reasons already of record, there would have been no motivation for combining the two references.

Claim 8 is further distinguishable over the applied combination of references. This is because Herman et al. only contemplates a method that allows for methylation-specific amplification of single fragments. While it may be *possible* to repeat this method with different single fragments until a certain percentage of the genome is reached, Herman et al. does not teach or suggest doing so. For example, to reach a coverage of 0.01% of the genome with the method of Herman et al., 1000 different fragments (assuming 300 bp average length) would have to be produced in as many different reactions. However, Herman et al. does not teach or suggest the generation of as many as 1000 fragments in one step, as does the present method.

Accordingly, for at least the above reasons, the foregoing rejection should be withdrawn.

Claim 6 stands rejected under 35 U.S.C. 103(a) "over Herman et al. (U.S. Patent 6,265,171 B1) (July 24, 2001) in view of Koster (U.S. Patent 5,605,798) (February 25, 1997) further in view of Katouzian-Safadi et al. (Biochimie, (1994), Vol. 76, (2), pages 129-132)." In support of the rejection the Patent Office states the following:

> Herman et al. in view of Koster teach the method of claims 1-5, and 7-23 as described above.
>
> Herman et al. in view of Koster do not teach the method, further characterized in that the oligonucleotide bound to the surface contain 5-bromouracil structural units.

> Katouzian-Safadi et al. teach the method, further characterized in that the oligonucleotide bound to the surface contain 5-bromouracil structural units (Summary and Results Section).
>
> It would have been *prima facie* obvious to one having ordinary skill in the art at the time the invention was made to combine and substitute the oligonucleotide containing 5-bromouracil structural units of Katouzian-Safadi et al. in the mass spectrometric method of detection of methylated nucleic acid using agents which modify unmethylated cytosine and distinguish modified methylated and unmethylated nucleic acids of Herman et al in view of Koster since Katouzian-Safadi et al. state, "The substitution of thymine by 5-bromouracil in DNA increases the photocrosslinking yield, and reduces the direct damages to both DNA and protein (Summary, second sentence)." An ordinary practitioner would have been motivated to combine and substitute the oligonucleotide containing 5-bromouracil structural units of Katouzian-Safadi et al. in the mass spectrometric method of detection of methylated nucleic acid using agents which modify unmethylated cytosine and distinguish modified methylated and unmethylated nucleic acids of Herman et al. in view of Koster in order to achieve the express advantage, as noted by Katouzian-Safadi et al., of the substitution of thymine by 5-bromouracil in DNA, which increases the photocrosslinking yield, and reduces the direct damages to both DNA and protein.

Applicant respectfully traverses the foregoing rejection. Claim 6 depends from claim 1. Claim 1 is patentable over the combination of Herman et al. and Köster for at least the reasons set forth in the previous rejection. Katouzian-Safadi et al. fails to cure all of these deficiencies. Consequently, the applied combination of Herman et al., Köster and Katouzian-Safadi et al. does not render obvious claim 6. Moreover, Applicant respectfully submits that one of ordinary skill in the art would not have been motivated to use the teachings of Katouzian-Safadi et al. in the manner proposed by the Patent Office. The alleged advantages recited in Katouzian-Safadi et al. are too general and remote to the present set of facts to have motivated a person of ordinary skill in the art to make the proposed modification.

Accordingly, for at least the above reasons, the foregoing rejection should be withdrawn.

Claim 24 stands rejected under 35 U.S.C. 103(a) "over Herman et al. (U.S. Patent 6,265,171 B1) (July 24, 2001) in view of Koster (U.S. Patent 5,605,798) (February 25, 1997) further in view of Stratagene Catalog (1988, Page 39)." In support of the rejection, the Patent Office states the following:

> Herman et al. in view of Koster expressly teaches the claims 1-5, and 7-24 as described above in detail.
>
> Herman et al. in view of Koster do not teach the motivation to combine all the reagents for identification of cytosine methylation patterns in a genomic DNA samples in the form of a kit.
>
> Stratagene catalog teaches a motivation to combine reagents into kit format (page 39).
>
> It would have been prima facie obvious to one having ordinary skill in the art at the time the invention was made to combine a suitable container, a sample holder for a mass spectrometer, all the reagents for identification of cytosine methylation patterns in a genomic DNA samples, as taught by Herman et al. in view of Koster into a kit format as discussed by Stratagene catalog since the Stratagene catalog teaches a motivation for combining reagents of use in an assay into a kit, "Each kit provides two services: 1) a variety of different reagents have been assembled and pre-mixed specifically for a defined set of experiments. Thus one need not purchase gram quantities of 10 different reagents, each of which is needed in only microgram amounts, when beginning a series of experiments. When one considers all of the unused chemicals that typically accumulate in weighing rooms, desiccators, and freezers, one quickly realizes that it is actually far more expensive for a small number of users to prepare most buffer solutions from the basic reagents. Stratagene provides only the quantities you will actually need, premixed and tested. In actuality, the kit format saves money and resources for everyone by dramatically reducing waste. 2) The other service provided in a kit is quality control". (page 39, column 1).

Applicant respectfully traverses the foregoing rejection. Claim 24 depends from claim 1. Claim 1 is patentable over the combination of Herman et al. and Köster for at least the reasons set forth above. Stratagene fails to cure all of these deficiencies. Consequently, the applied combination of Herman et al., Köster and Stratagene does not render obvious claim 24. Moreover, Applicant respectfully submits that one of ordinary skill in the art would not have been motivated to use the teachings of Stratagene in the manner proposed by the Patent Office. The alleged advantages recited in Stratagene are too general and remote to the present set of facts to have motivated a person of ordinary skill in the art to make the proposed modification. The Patent Office's argument, taken to its logical endpoint, would render any reagent kit obvious on the basis of Stratagene. This obviously cannot be the correct outcome.

Accordingly, for at least the above reasons, the foregoing rejection should be withdrawn.

In conclusion, it is respectfully submitted that the present application is in condition for allowance. Prompt and favorable action is earnestly solicited.

If there are any fees due in connection with the filing of this paper that are not accounted for, the Examiner is authorized to charge the fees to our Deposit Account No. 11-1755. If a fee is

required for an extension of time under 37 C.F.R. 1.136 that is not accounted for already, such an extension of time is requested and the fee should also be charged to our Deposit Account.

Respectfully submitted,

Kriegsman & Kriegsman

By: _____

Edward M. Kriegsman
Reg. No. 33,529
665 Franklin Street
Framingham, MA 01702
(508) 879-3500

Dated: March 26, 2003

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Box AF, Commissioner for Patents, Washington, D.C. 20231 on March 26, 2003

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Edward M. Kriegsman
Reg. No. 33,529
Dated: March 26, 2003